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(71) Applicant *(for all designated States except US)*: NUTRON LIMITED [-/--]; Charwell House, Braye Road Industrial Estate, Vale, Guernsey (GB).

(71)(72) Applicant and Inventor: PRICE, Thomas, Gairdner [GB/GB]; Keepers Cottage, Well Hall Lane, Betchworth, Surrey RH3 7HH (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : STOAKES, Ian [GB/GB]; 4 Shere Court, Hook Lane, Shere, Surrey GU5 9QH (GB).

(74) Agents: TRIBE, Thomas, Geoffrey et al.; F.J. Cleveland & Company, 40/43 Chancery Lane, London WC2A 1JQ (GB).

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METHOD OF DETERMINING THE ALLERGENIC RESPONSE OF A SAMPLE OF MAMMALIAN BLOOD TO AN ANTIGEN

DESCRIPTION

The present invention relates to a method of determining the allergenic response of a sample of mammalian blood to an antigen, particularly a food antigen. The invention also embraces an *in vitro* method of diagnosis for detecting an allergenic response to an antigen in a patient.

Some patients are allergic to certain foods. The particular foods vary from patient to patient, and although the mechanism of the allergenic response to food allergens is not fully understood, it is thought that allergenic reactions to foods may in some patients be at least partly responsible for symptoms as diverse as migraine, asthma, eczema and arthritis.

A particular disease in which food intolerance may be implicated is obesity. In some patients, obesity is a result of fluid retention. It is thought that in some patients an allergenic response to certain food antigens may give rise to an increase in the permeability of their blood capillaries; the capillaries become leaky, and water in the blood escapes into tissue juxtaposed the capillaries. As a result, the patient may feel thirsty and drink more liquid than normal to compensate. However, the additional consumed fluid ultimately also escapes from the capillaries, and the patient becomes "fluid retaining" and puts on weight.

Once an allergenic response to a particular food has been diagnosed in a patient, an effective treatment is the elimination of the food from the patient's diet. A typical daily diet, however, includes many different types of food; there can be considerable difficulty therefore in determining which foods cause an allergic response in a particular patient and should, therefore, be avoided.

Furthermore, there is a general prejudice among medical professionals and the public alike against the recognition of a causal link between the symptoms mentioned above and allergic intolerance.

In consequence testing for food intolerance has hitherto been confined outside mainstream general medical practice, and has included techniques for which the scientific basis has not been satisfactorily proven.

Testing for food intolerance has conventionally been carried out by a "skin-prick" test in which a patient is pricked with a sterilized point carrying a small quantity of a food antigen. The patient's response to the food antigen is monitored visually to determine whether or not an intolerance is exhibited. Although a patient can be tested for intolerance to five or more antigens simultaneously by subjecting the patient to a plurality of pricks at different skin locations, the "skin-prick" test is time consuming, and testing for tens of foods can take many weeks for a single patient. The prick test also suffers from the disadvantage that visual monitoring is required;

small and/or transient reactions may therefore be unnoticed.

It has been observed that the total number of leucocytes in a sample of blood from a patient may decline if the sample is challenged with an antigen to which the patients exhibits an allergenic reactions.

US-A-4,614,722 discloses a method and apparatus for the determination of the degree of reaction between a suspected allergen and a blood sample; the method involves comparing the total number of white blood cells present in the sample before and after reaction with the suspected allergen.

It is now thought that the various symptoms which may result from an allergenic response in a patient to one or more foods may be caused by the release of granules from one or more of the granulocyte populations in the blood of the patient, typically the neutrophils, into the patient's blood stream. In particular, it is thought that certain food allergens may interact with the granulocytes in the patient's blood causing some of the granulocytes to rupture and release granules

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into the blood stream. It will be appreciated by a person skilled in the art that the chemicals normally released by a granule in the cytoplasm of a granulocyte may, if released directly into the blood stream of a patient, cause inflamation or damage to tissue which may, in turn, lead to the symptoms associated with those medical conditions thought to be affected by an intolerance to foods.

According to one aspect of the present invention therefore there is provided a method of determining the allergenic response of a sample of mammalian blood to an antigen; which method comprises the steps of:-

a) adding the antigen to the blood sample to challenge the white blood cells in the blood sample;

b) allowing the blood sample/antigen mixture to incubate;

c) thereafter probing the mixture to determine the amount of degranulation of one or more of the granulocyte populations in the blood sample

means for adding the antigen to the blood sample;

means for probing the blood sample/antigen mixture to determine the amount of degranulation of one or more of the granulocyte populations in the blood sample relative to antigen-free control blood sample; and

means for comparing the amount of degranulation with a statistical mean degranulation derived from testing a plurality of other blood samples thereby to determine whether the amont of degranulation is significant as compared with the said mean degranulation.

Typically, the blood sample/antigen mixture may be probed to determine the amont of degranulation in the neutrophil population.

The amount of degranulation may be determined in some embodiments by directly measuring the number of granules leaked by the granulocytes in the sample into the blood plasma. Alternatively, the amount of degranulation may be determined indirectly by

eosinophils. Each aliquot may be suspended in an electrolytic diluent and disposed in a transducer chamber having a partition dividing the transducer chamber into two regions. The partition may have a small aperture extending therethrough between the two regions; each region may be provided with an electrode. The size of the aperture may be of the same order as the size of the white blood cells in the blood sample; the electrolytic diluent may be selected to have a significantly different electrical conductance from said white blood cells.

The white blood cells in each aliquot may be aspirated through the aperture from one region of the transducer chamber to the other region. A substantially constant electrical current may be applied across the electrodes; as a white blood cell passes through the aperture the voltage between the electrodes may change transiently by a detectable amount; said voltage may be monitored continuously during aspiration of the cells to count the number of cells passing through the aperture.

lymphocytes, granulocytes and monocytes may show up in different regions of the scattergram as a result of their different overall volume to nuclear/granule volume ratios. Based on experience, therefore, "lymphocyte", "granulocyte" and "monocyte" regions of the scattergram may be delineated by applying calculated "discriminator" lines; the total number of cells represented in each region may then be counted.

The apparent neutrophil population in the blood sample may be calculated by subtracting the counted numbers of basiphils and eosinophils in the second and third aliquots from the calculated apparent granulocyte population in the first aliquot.

In accordance with the present invention it will be appreciated that a degranulated granulocyte may appear under d.c. and r.f. analysis as described above as a "monocyte" or lymphocyte". Thus, in one aspect of the invention, degranulation of one or more of the granulocyte populations in the blood sample may be detectable as an apparent increase in the number of

In some embodiments a plurality of food antigens may be selected which include sufficient foods to constitute a complete and balanced diet.

The foods antigens may be presented to the blood sample in suspension. The suspension medium may be glycerol or a benzoyl solvent. In one embodiment, glycerol suspensions of food antigens obtained from Messrs. Dome/Hollister-Stier may be used; the antigen suspensions may be of the type typically used for conventional 'skin-prick' testing.

The food antigen/glycerol suspension may be further diluted. The dilutant may be a saline solution, typically saline BP.

The food antigen suspension may include a small proportion of human serum albumen (HSA); about 0.01% to 0.05% HSA may be used, typically 0.03%.

Where food antigens obtained from Messrs. Hollister-Stier are used, the glycerol suspension may be diluted with saline BP in the ratio about 1:9, but it will be understood that the present invention

The respective increase and decrease in the apparent monocyte/lymphocyte and granulocyte populations of the blood sample for a given food antigen may be compared respectively with the mean increase and decrease obtained from a plurality of blood samples. A significant increase or decrease respectively as compared with the obtained mean, as background, may indicate an intolerance to the antigen concerned. The obtained mean increase or decrease in the monocyte/lymphocyte and granulocyte populations respectively may vary from food antigen to food antigen, but typically a decrease of about 5% - 10% in, say, the neutrophil population may indicate an intolerance.

Once an intolerance to a particular food antigen has been determined for a given blood sample, treatment of the patient may be effected by advising the patient to eliminate the food from his/her diet.

Alternatively, in accordance with one aspect of the present invention, the patient may limit his/her diet to foods which do not apparently provoke an allergenic reaction; foods not tested may be reintroduced into

In the drawings:-

Figure 1 is a scattergram of an antigen-free control blood sample.

Figure 2 is an histogram of the blood sample of Figure 1.

Figure 3 is a scattergram of a blood sample from the same donor as the sample of Figure 1 after challenge with a food antigen.

Figure 4 is a histogram corresponding to the scattergram in Figure 3.

Figure 5 is a scattergram of a different sample of blood from the same donor as Figures 1 and 3, which different sample has been challenged with a different food antigen.

Figure 6 is a histogram which corresponds to the scattergram in Figure 5.

cod	almond
crab	brazil nut
haddock	cashew
mackerel	coconut
plaice	hazel nut
prawn	peanut
salmon	olive
sardine	sesame seed
sole	sunflower seed
trout	walnut/English
tuna	

GRAINS	FRUIT
barley	apple
maize/corn	avocado
malt	banana
oats	blackcurrent
rice	date
rye	grape
wheat	grapefruit
	lemon
	mango

egg plant/aubergine
garlic
green pepper (bell)
leek
lettuce
onion
parsnip
potato
radish
spinach
sprout
tomato
turnip

honey
saccharine
sugar, beet
sugar, cane

ADDITIVES

black/white pepper mix
monosodium glutamate
salicylates
tea

A sample of blood was taken from a patient and divided into 100 portions. Each portion was mixed with one of the food antigen mixtures in a ratio one part blood to one part food antigen mixture. One portion of blood was mixed with an antigen-free mixture containing one part glycerol, nine parts saline BP and 0.03% HSA as a control.

between first and second electrodes disposed respectively in the first and second regions of the transducer chamber; the aperture has a diameter of the same order of magnitude as the size of the white blood cells in the aliquot, thus as a blood cell passes through the aperture the resistance of the electrical path between the electrodes in the chamber increases significantly; in consquence the voltage drop across the electrodes increases transiently by a detectable amount.

On aspirating the second and third aliquots, a substantially constant direct current (d.c.) is applied between the electrodes; the transient change in d.c. voltage as a blood cell is aspirated through the aperture being proportional to the total cell volume. On aspirating the first aliquot, a substantially constant direct current and a substantially constant radio frequency alternating current (r.f.) are applied. The transient change in the r.f. voltage as a leucocyte passes through the aperture is proportional to the total nuclear and cytoplasmic granule volume and density of the leucocyte.

generates a histogram (see, for example, Figures 2, 4 and 6 of the Drawings hereto) which shows the relative numbers of white blood cells counted/calculated in each cell population.

The food antigen-free control sample was supplied to the haematology analyser, and the relative concentrations of the respective leucocyte populations in the control are set out in Table 1 below.

TABLE 1

LEUCOCYTE	PERCENT (%)
neutrophil	45.2
lymphocyte	44.7
monocyte	8.0
eosinophil	1.6
basiphil	0.5

TABLE 2

LEUCOCYTE	PERCENTAGE (%)
neutrophil	41.1
lymphocyte	42.2
monocyte	14.5
eosinophil	1.7
basiphil	0.5

TABLE 3

LEUCOCYTE	PERCENTAGE (%)
neutrophil	16.2
lymphocyte	56.6
monocyte	18.5
eosinophil	3.5
basiphil	5.2

and 4, on the other hand, indicate an apparent non-reaction, indicating that the patient is not significantly sensitive to that particular food antigen.

It will be appreciated that the various leucocyte populations in blood vary naturally with time. Thus, it is important that the blood/food antigen mixtures and the control mixture are probed using the haematology analyser at as close to the same time as possible. Furthermore, depending on the concentration of the food antigens and suspension media used, small changes in the granulocyte/monocyte populations may be detected with any of the food antigens, and it is important, therefore, to determine which of the changes indicates a positive reaction to a particular food antigen. The results obtained for a patient, therefore, are compared with the mean leucocyte population changes for a particular food-antigen derived from a plurality of blood samples taken from different patients. For each food-antigen, a marked change in one of the leucocyte populations as compared with the mean may indicate an intolerance to the food antigen. Thus, a statistically significant decline